Finally, this is a physically and intuitively appealing characterization of black hole based on balance between gravitational charge and field energy. An excellent example of this kind of consideration is the escape velocity argument, which though gives the right result follows from an invalid relation. It only refers to escape of particles from the gravitational field and not to one-way character of a closed 2-surface, which is the characteristic feature of the horizon. The horizon marks irresistability of gravitational pull through gravitational charge, as well as one-way character, no escape out of closed surface, through space curvature caused by the field energy. These two features must define the same surface, as the photon is the limiting case of an ordinary particle.

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The use of RAPD in assessing genetic variability in *Andrographis paniculata* Nees, a hepatoprotective drug

P. Padmesh, K. K. Sabu, S. Seeni and P. Pushpangadan

Plant Biotechnology Division, Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, India

RAPD analysis was done to determine intraspecific variability in *Andrographis paniculata*, a popular antipyretic and hepatoprotective drug used in traditional medicine in India. The accessions collected from parts of India and south-east Asia on molecular analysis revealed moderate variation within the species. Similarity measurement using UPGMA followed by cluster analysis resulted in 5 major groups based on geographical distribution that generally reflected expected trends between the genotypes. There were also important exceptions like AP-48, an accession from Thailand showing close resemblance to AP-38 collected from Tamil Nadu and AP-29 from Assam significantly diverse from the rest of the native genotypes. The results indicated that RAPD could be effectively used for genetic diversity analysis in wild species of prospective value as it is reliable, rapid and superior to those based on pedigree information.

INDIA being one of the twelve megadiversity centres has immense biotic wealth marked by remarkable ecosystem, species and genetic diversity. This rich biological diversity is matched equally by rich cultural diversity and health traditions. Over 7000 species out of an estimated 17,000 higher angiosperms recorded from India are reportedly used for medicinal purposes[1]. For efficient conservation and management of medicinal plant diversity, the genetic composition of species collected from different phytogeographical regions needs to be assessed. While there are a few commendable efforts to study the genetic variation within populations of selected medicinal plant species based on isozyme profile in different regions of distribution[2], a broad based analysis of genetic diversity between and within the species is still lacking. Molecular analysis of intraspecific variation in particular may find application in resolving disputes of taxonomic identities, relations and authentication of the species, developing a comprehensive database of genetic variability in the species for future reference and protection of genetic diversity of the species, identification of useful genotypes that could be developed as cultivars for field trials and sustainable utilization through formulation of standard drugs free from batch to batch variations. Since a vast majority of Indian population is dependent on traditional medicine for primary health care, and as against the recent revival of interest in plant medicines across the globe and consequent pressure on precious herbal resources, it makes sense to rationalize the use of medicinal plants through scientific screening and validation. In the present study we provide evidence through RAPD assay for the occurrence of genetic variation in *Andrographis paniculata* the Kalmegh of Ayurveda, reputed for its antipyretic and hepatoprotective properties, wide distribution and high adaptability in different phytogeographical zones of south-east Asia. The genus *Andrographis* as a whole is of potential significance to India as 25 out of 28 species in the world are distributed in India with 23 of them occurring in the peninsular region[3].

A germplasm collection of 52 accessions was organized from different parts of India, Thailand, Malaysia and Indonesia and maintained under uniform growth conditions. Of these 15 accessions, those displaying interesting morphological and phytochemical variations were selected (data not shown) for further investigations (Figure 1). Total genomic DNA from the young leaves of the plants was isolated following the modified Murray and Thompson method using CTAB. Extraction buffer contained 1.2% PVP-40T (mol wt 40,000, Sigma, USA) to remove high phenolic contaminants and double $CHCl_3$ extraction at 10,000 rpm helped to remove poly-

Figure 1. Three morphovariants among the 15 accessions of *A. paniculata* used in the present study.

Table 1. List of primers, their sequence and products generated through amplification

Primer code	Primer sequence 5′-3′	Total no. of bands	No. of polymorphic bands
OPZ-01	TGTGTGCCAC	6	4
OPZ-02	CCTACGGGGA	6	3
OPZ-04	AGGCTGTGCT	11	11
OPZ-06	GTGCCGTTCA	9	9
OPZ-08	GGGTGGGTAA	12	10
OPZ-10	CCGACAAACC	8	5
OPZ-12	TCAAGGGGAC	8	8
OPZ-16	TCCCCATCAC	6	6
OPAW-03	CCATGCGGAG	4	3
OPW-05	CTGCTTCGAG	3	2
Total no. of bands		73	61
Mean per primer		7.3	6.1

saccharides. After ethanol precipitation, DNA was resuspended in 100 µl of I × TE buffer (pH 8.0). RAPD assay was carried out in 25 µl reaction mixture containing 2.5 µl 10 × amplification buffer (500 mM KCl, 100 mM Tris-HCl, 1.0% Triton X-100 and 15 mM $MgCl_2$), 100 µl each of dATP, dGTP, dCTP and dTTP, 1.0 U of Taq DNA polymerase (Promega, USA), 15 pmoles of 10-mer primer (Operon Technologies Inc, USA) and 50 µg of genomic DNA. Amplification was performed in PTC 100 Thermal Cycler (MJ Research Inc, USA). The sequential steps involved: 1 cycle of 2 min at 93°C, 2 min at 35°C and 2 min at 72°C followed by 44 cycles of 1 min at 93°C, 1 min at 36°C and 2 min at 72°C. The last cycle was followed by 10 min extension at 72°C.

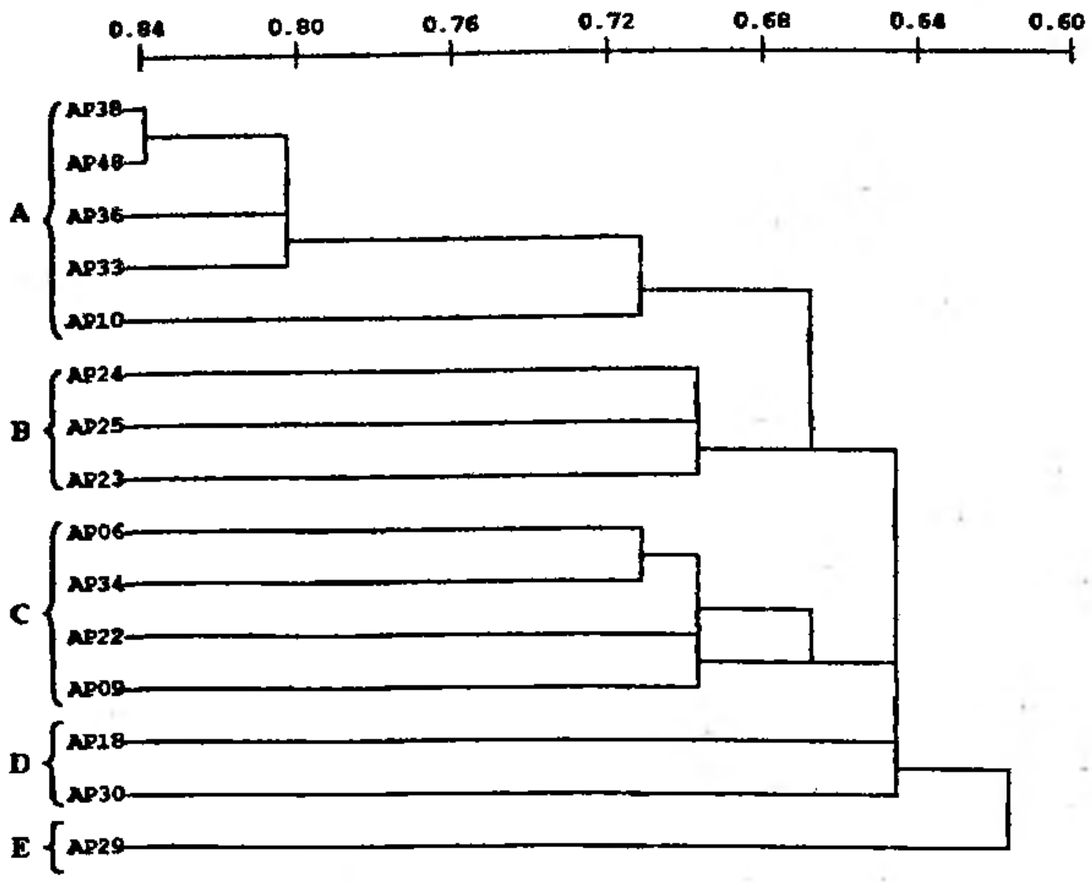

Figure 2. Dendrogram obtained from RAPD analysis using UPGMA.

The amplified products were resolved in 1.2% agarose gel (1 × TBE) followed by EtBr staining and the bands detected were photographed. Amplified products which were reproducible and consistent in performance were chosen for scoring of shared and unshared bands. The presence of a particular band was denoted as 1 and absence as 0. Out of the 73 amplified products using 10 primers, 61 were found to be polymorphic (83.56%) with an average of 6.1 bands per primer (Table 1). However, there were no genotype/accession-specific products. The number of bands produced per primer ranged from 12 (OPZ-08) to 3 (OPAW-05), with an average of 7.3 bands per primer and the products ranged in size from 310 bp to 3500 bp. Multivariate analysis to detect genetic similarity coefficient was performed following Nei and Li's method[4] and the similarity matrix obtained is shown in Table 2. Cluster analysis was performed based on the matrix obtained using the computer program PCO3D (version 1.2)[5] and the clusters were represented in the form of a dendrogram (Figure 2).

The present study and similar studies on *Lotus*, sweet potato, oil palm and potato[6–9] suggest that RAPD is more appropriate for analysis of genetic variability in closely-related genotypes. The level of genetic variability detected is moderately high in *A. paniculata* with 6.1 polymorphisms revealed per primer. The coefficient similarity values ranging from 0.38 to 0.89 indicate that the distribution of variation is diverse. Although *A. paniculata* seems to be an inbreeder (Sabu, K. K, Padmesh, P. and Seeni, S., unpublished), the mean coefficient similarity of 0.60 substantiates the moderately broad distribution of genetic variability which can be attributed to the broad genetic base in the origin of the species. The dendrogram obtained through cluster analysis revealed 5 major clusters A, B, C, D and E

Table 2. Similarity matrix of 15 accessions of *A. paniculata*

	AP-06	AP-09	AP-10	AP-18	AP-29	AP-22	AP-23	AP-24	AP-25	AP-30	AP-33	AP-34	AP-36	AP-38	AP-48
AP-06	1.0														
AP-09	0.84	1.0													
AP-10	0.67	0.65	1.0												
AP-18	0.61	0.63	0.63	1.0											
AP-29	**0.45**	**0.42**	**0.51**	**0.29**	**1.0**										
AP-22	0.73	0.65	0.78	0.60	**0.44**	1.0									
AP-23	0.75	0.75	0.72	0.67	**0.23**	0.63	1.0								
AP-24	0.64	0.66	0.63	0.60	**0.37**	0.70	0.63	1.0							
AP-25	0.70	0.74	0.63	0.37	**0.63**	0.73	0.79	0.71	1.0						
AP-30	0.71	0.57	0.66	0.63	**0.45**	0.51	0.66	0.71	0.53	1.0					
AP-33	0.62	0.67	0.74	0.72	**0.40**	0.63	0.76	0.72	0.80	0.56	1.0				
AP-34	0.56	0.45	0.43	0.44	**0.44**	0.41	0.41	0.39	0.38	0.52	0.42	1.0			
AP-36	0.63	0.66	0.81	0.70	**0.41**	0.63	0.77	0.61	0.67	0.60	0.85	0.38	1.0		
AP-38	0.70	0.66	0.77	0.77	**0.42**	0.71	0.77	0.73	0.76	0.66	0.89	0.45	0.89	1.0	
AP-48	0.60	0.69	0.75	0.69	**0.49**	0.62	0.75	0.64	0.72	0.63	0.81	0.45	0.86	0.93	1.0

which in fact is in broad agreement with the geographical distribution of the genotypes with the exception of AP-48 and AP-29. Cluster A with five genotypes from different parts of Tamil Nadu (AP-38, AP-36, AP-33 and AP-10) with the exception of AP-48 (Thailand) shared a similarity value ranging from 0.84 to 0.71 with an average of 0.78. Inclusion of AP-48 in this cluster is quite interesting since it is geographically distant from the rest. It showed close resemblance to AP-38 (93%) from Alagar hills of Tamil Nadu and least with AP-29 (49%) from Assam. It is possible that genotypes from different geographical regions can be genetically similar to genotypes with immediate spatial relationships. This association between distantly located genotypes may be attributed either to the unique and broad genetic base of the species which enable it to maintain and exist in different gene combinations or to the possible Indian provenance of AP-48 since *A. paniculata* is an introduced species in Thailand. Clusters B and C having genotypes from local and distant localities of Kerala (AP-23, AP-24, AP-25, AP-06, AP-34, AP-22 and AP-09) respectively shared almost 71% similarity and this association between genotypes from contiguous regions may be the result of similar agroclimatic conditions or due to seed movement and gene flow. Accessions from Pune and Mangalore (AP-18 and AP-30) together constituted cluster D with the apparent isolation of AP-29 as a separate cluster E. Although AP-29 is a native genotype, it is found to be significantly diverse from the rest displaying a maximum similarity coefficient of 0.51 with AP-10 and minimum of 0.23 with AP-23. This may be due to lot of missing data points as the number of primers and amplified products analysed were relatively less. Although the data presented here are not conclusive to infer the phenetic relationship between the various accessions, they reflect the utility of RAPD in the analysis of genetic variability distribution within this important medicinal herb. We are currently involved in improving this analysis by scoring additional loci using the available 10-mer primers to find correlation between the observed chemical variation and RAPD profile and also to increase the number of accessions for wide and comprehensive information about the pattern of genetic variation distribution in this species.

In practice, better understanding of distribution of genetic variation at the intraspecific level would help identify superior genotype(s) for cultivar upgradation as well as to evolve strategies for the establishment of effective *in situ* and *ex situ* conservation programmes. Although such empirical determination of genetic diversity can be obtained by evaluating morphological, physiological and biochemical traits, this information is not available for *A. paniculata* and to our understanding, this is probably the first report which deals with the analysis of genetic diversity at the molecular level in a native medicinal plant.

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